“Exploring drug efficacy in experimental Chagas disease using highly sensitive bioluminescence imaging”

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Improving health worldwide  www.lshtm.ac.uk
Inoculate parasites
Inject d-luciferin
Recover and return to cage

Anaesthetize
Acquire images

In vivo imaging
Validating the model

The limit of detection

Correlation between bioluminescence and parasite burden

Profile of expression

Correlation with qPCR
Visualising chronic *Trypanosoma cruzi* infection

**Chronic  *T. cruzi* infection: dynamic not latent!**

- **Day 125**
- **Day 127**

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**Graph:**
- **Y-axis:** Total flux (pA)
- **X-axis:** Day post-infection

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**Legend:**
- **Ventral**
- **Dorsal**

**Scale:**
- 1x10^7
- 1x10^4
The GI tract is the major site of parasite persistence in chronic infections.
Inducing relapse by immunosuppression

CYP+0  CYP+3  CYP+6  CYP+9  CYP+13  CYP+16

Day 0  Day 6  Day 9

cyclophosphamide
Summary - 1

• BLI can be used to track chronic *T. cruzi* infections in mouse models

• The GI tract is the major site of parasite persistence in chronic infections

• Chronic *T. cruzi* infections are highly focal and spatiotemporally dynamic

• BLI can be used as a tool to:
  
  Phenotype genetically engineered parasite mutants
  
  Investigate the mechanisms of pathogenesis
  
  Assess drug efficacy
**Chronic stage infection: Comparing benznidazole and posaconazole**

### Drug and Treatment Summary

<table>
<thead>
<tr>
<th>Drug</th>
<th>Disease state</th>
<th>Treatment time</th>
<th>Daily dose</th>
<th>Number cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benznidazole</td>
<td>chronic</td>
<td>20 days</td>
<td>100 mg/kg</td>
<td>5/5</td>
</tr>
<tr>
<td>Benznidazole</td>
<td>acute</td>
<td>20 days</td>
<td>100 mg/kg</td>
<td>5/5</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>chronic</td>
<td>20 days</td>
<td>20 mg/kg</td>
<td>0/9</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>acute</td>
<td>20 days</td>
<td>20 mg/kg</td>
<td>3/19</td>
</tr>
</tbody>
</table>

**Non-treated**

- Benznidazole (100 mg/kg) (days 74 - 94)
- Cyclophosphamide (days 113, 118, 128)

**Benznidazole (100 mg/kg) (days 74 - 94)**

- Cyclophosphamide (days 113, 118, 128)

**Posaconazole (20 mg/kg) (days 74 - 94)**

- Cyclophosphamide (days 113, 118, 128)
Parasite recrudescence following posaconazole treatment

Treated days 14 – 33
Immunosuppressed
Imaged day 74

Treated days 74 – 93
Immunosuppressed
Imaged day 147

9/16 display high level infection in adipose tissue

1/9 display high level infection in adipose tissue
Benznidazole cures chronic stage infections more readily than acute stage infections. 100 mg/kg qd for 5 days

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment (days)</th>
<th>Dose (mg/kg)</th>
<th>Cure rate Chronic</th>
<th>Cure rate Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benznidazole</td>
<td>20</td>
<td>100</td>
<td>100% (11/11)</td>
<td>93% (14/15)</td>
</tr>
<tr>
<td>Benznidazole</td>
<td>10</td>
<td>100</td>
<td>100% (15/15)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Benznidazole</td>
<td>5</td>
<td>100</td>
<td>100% (11/11)</td>
<td>0% (0/30)</td>
</tr>
</tbody>
</table>
Benznidazole cures chronic stage infections more readily than acute stage infections

30 mg/kg qd for 20 days

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment (days)</th>
<th>Dose (mg/kg)</th>
<th>Cure rate Chronic</th>
<th>Cure rate Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benznidazole</td>
<td>20</td>
<td>30</td>
<td>100% (6/6)</td>
<td>33% (2/6)</td>
</tr>
<tr>
<td>Benznidazole</td>
<td>10</td>
<td>30</td>
<td>67% (4/6)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Benznidazole</td>
<td>5</td>
<td>30</td>
<td>0% (0/6)</td>
<td>-</td>
</tr>
</tbody>
</table>
Does differential drug exposure account for disease stage-specific benznidazole efficacy?

100 mg/kg single oral dose

- acute stage
- chronic stage
- uninfected
Why is benznidazole more effective against the chronic stage?

CHRONIC

ACUTE
Do other nitroheterocycles show the same stage-specific profile?

100 mg/kg qd for 5 days

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<th>Drug</th>
<th>Treatment (days)</th>
<th>Dose (mg/kg)</th>
<th>Cure rate Chronic</th>
<th>Cure rate Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benznidazole</td>
<td>5</td>
<td>100</td>
<td>100% (11/11)</td>
<td>0% (0/30)</td>
</tr>
<tr>
<td>Nifurtimox</td>
<td>5</td>
<td>100</td>
<td>90% (9/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Fexinidazole</td>
<td>5</td>
<td>100</td>
<td>100% (8/8)</td>
<td>67% (4/6)</td>
</tr>
<tr>
<td>Fexinidazole sulfone</td>
<td>5</td>
<td>100</td>
<td>100% (7/7)</td>
<td>100% (15/15)</td>
</tr>
</tbody>
</table>
Pharmacokinetic parameters

Benznidazole

Nifurtimox

Fexinidazole

Fexinidazole sulfone

100 mg/kg single oral dose
• Posaconazole has limitations as a treatment for experimental Chagas disease

• Adipose tissue is an important site of parasite recrudescence following posaconazole treatment of acute stage infections

• Benznidazole cures chronic stage infections more effectively than acute stage infections

• At the same dose, fexinidazole sulphone is more effective than benznidazole as a curative treatment for acute stage *T. cruzi* infections

• PCR-based methodologies have a tendency to over-estimate cure rates
**In vivo imaging of other trypanosomatid infections**

*Trypanosoma brucei*  
GVR35 strain

*Leishmania donovani*  
(DD8 strain)


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